
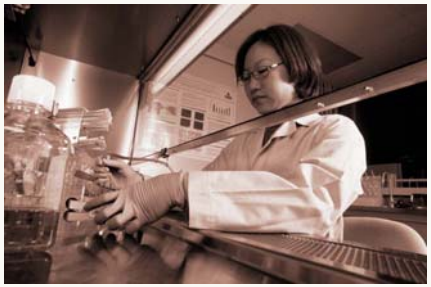


PURDUE UNIVERSITY

Predicting Cell Capture from Dilute Samples for Microfluidic Biosensors

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Department of Agricultural and Biological Engineering
Laboratory of Renewable Resources Engineering
Department of Statistics

AGRICULTURAL & BIOLOGICAL ENGINEERING

LORRE

Acknowledgements

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Food Borne Pathogens

BACTERIA	***L. monocytogenes***	***E. coli* O157:H7**
Incubation period	> 1-5 days	2-5 days
Symptoms	Gastroenteritis Stillbirth Death	Hemorrhagic colitis
Infectious dose	unknown	As few as 10 cells
Common sources	Soil, food processing environment	Feces/manure Raw meat

www.foodsafety.gov http://www.cfsan.fda.gov/~mow/intro.html

Listeria monocytogenes

Gram positive (1x 2 μm)

Grows at 1 to 45 ºC

Acid and salt tolerant

Annual cases >2,500;
Mortality 20-28%

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Rapid detection of bacteria

Technique / Features	Growth Based	Viability Based	Cell Comp. Based	Nucleic Acid-based
Live/Dead	Yes	Yes	No	No
Identification	Yes	No	Yes	Yes

How / why?

Using selective media

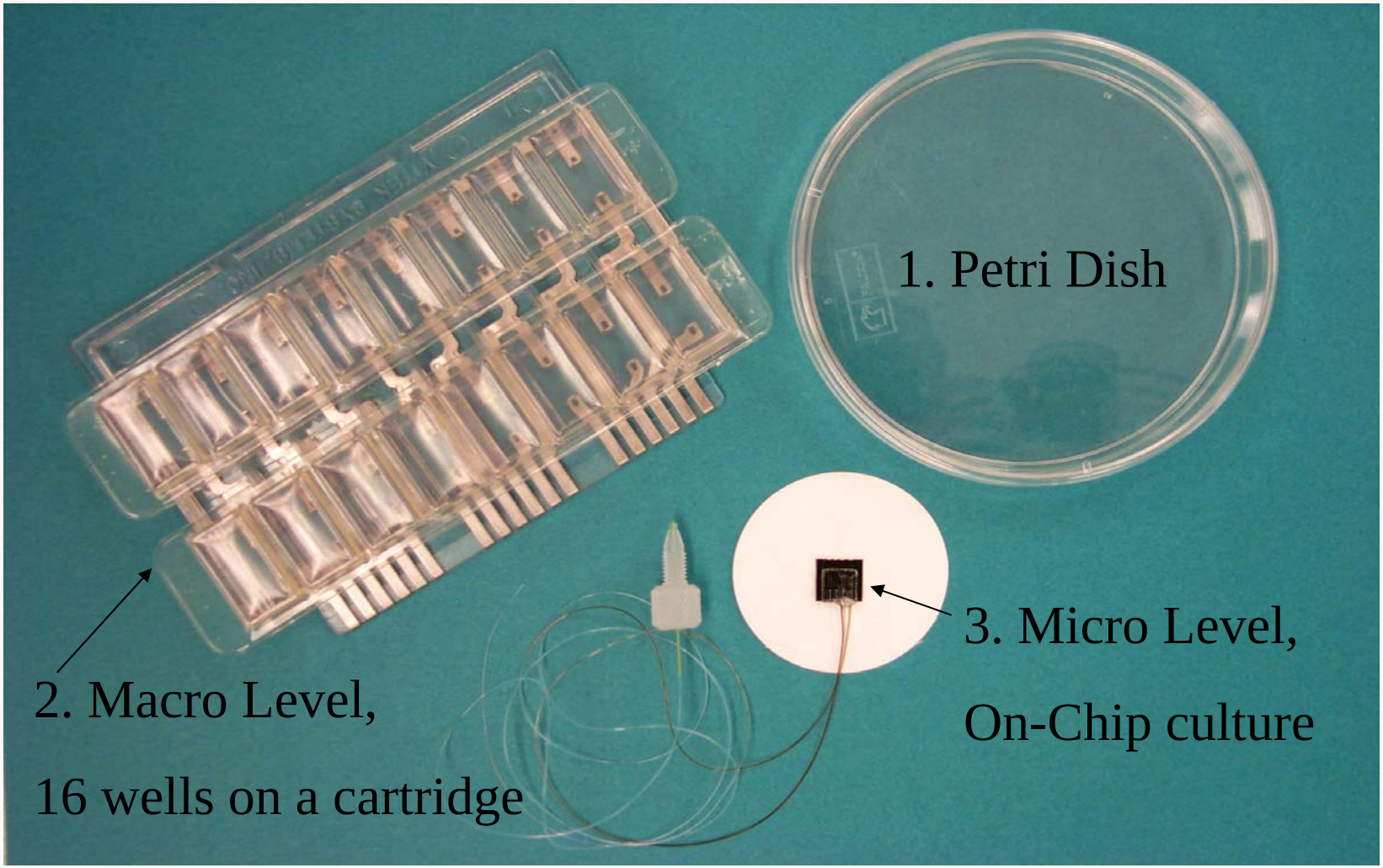
Growth steps required to provide live / dead information

Through antibody-based capture

Our research addresses **growth-based** and **cell-component based** approaches to provide **live / dead** and **ID** information

Adapted from M.R. Ladisch, presentation to USDA-ARS-EERC, 8 November 2004

Evolution of Growth Detection



Needs

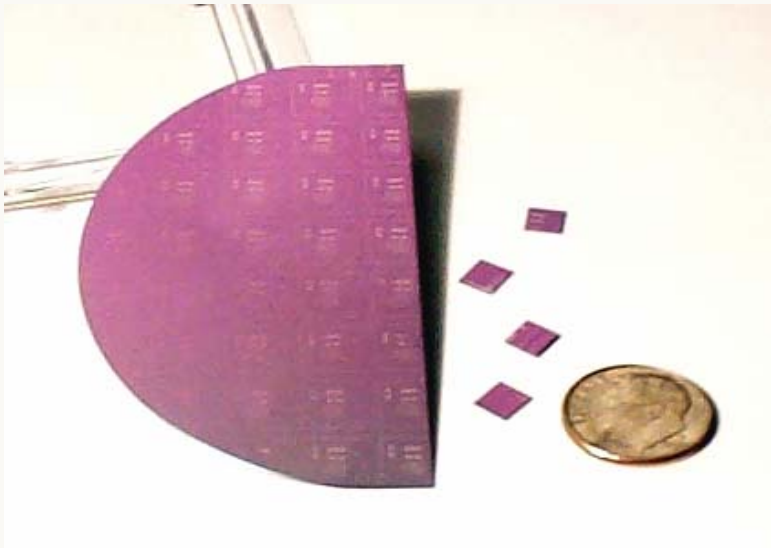
Rapid detection during food processing

Elapsed time between sampling and result (time to result) of 3 hours or less

Detection against background of other non-pathogenic organisms

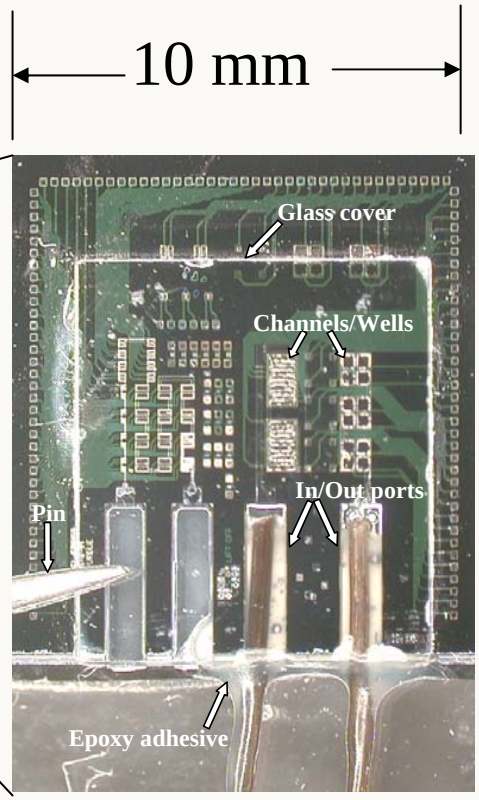
Pathogen Detection on Biochips

Microchips:
Can be made in large numbers

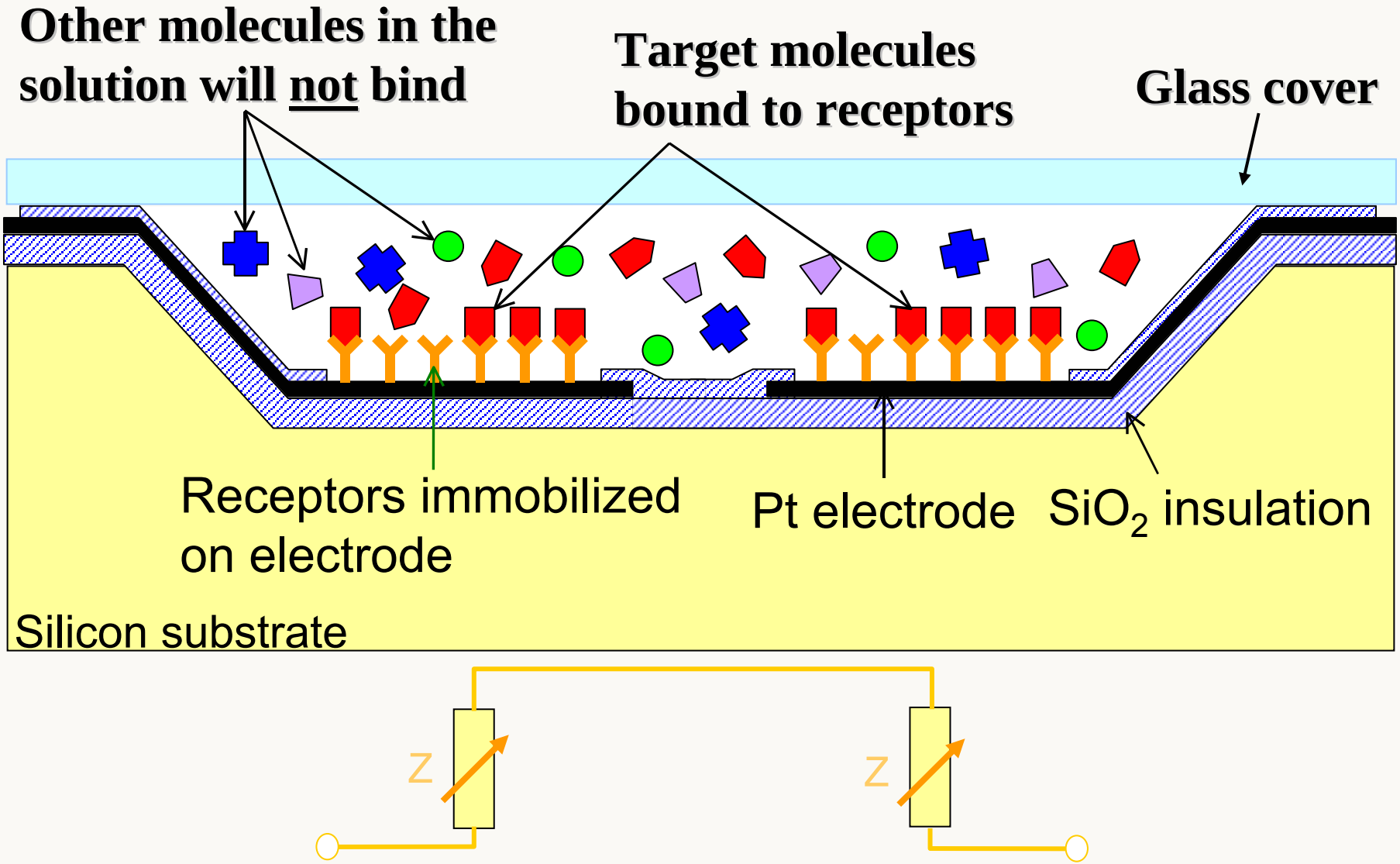


- Microchips for adsorption studies
- PECVD fabricated oxide layer
- SiO_2 with Pt patterns

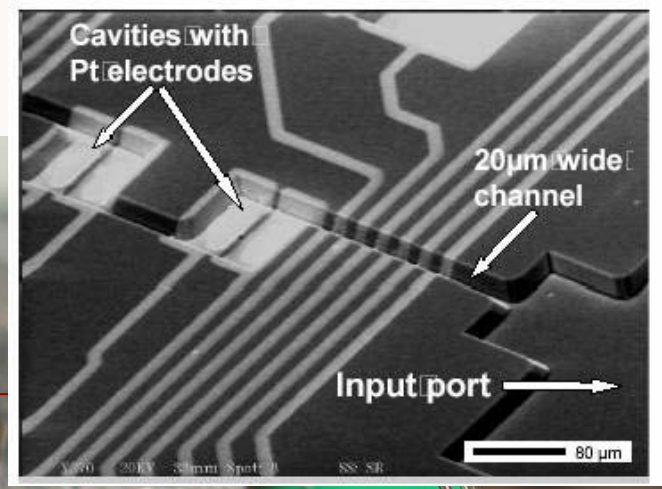

Rashid Bashir, ECE
Arun Bhunia, Food Sci
Paul Robinson, BMS, BME
Jennifer Sturgis, BMS
Rafael Gomez, ECE

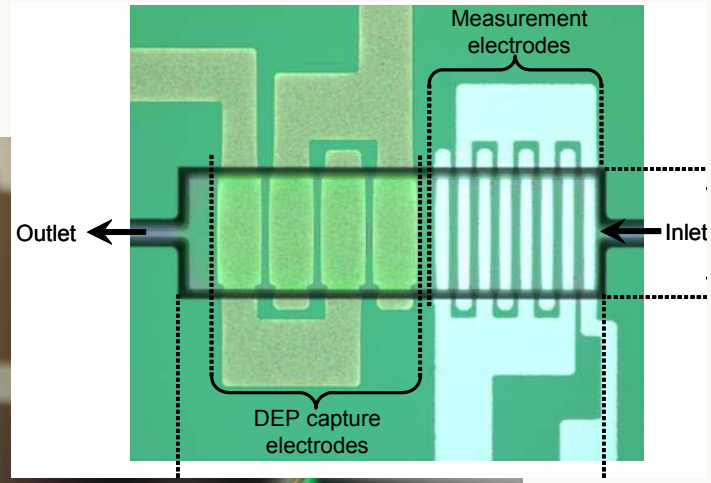

10 mm
Glass cover
Channels/Wells
In/Out ports
Pin
Epoxy adhesive

Protein Biochip Technology Platform



Micro-fabricated Biochip for Bacterial Cell Culture





Microscope Objective

Micro-fluidic Tubes

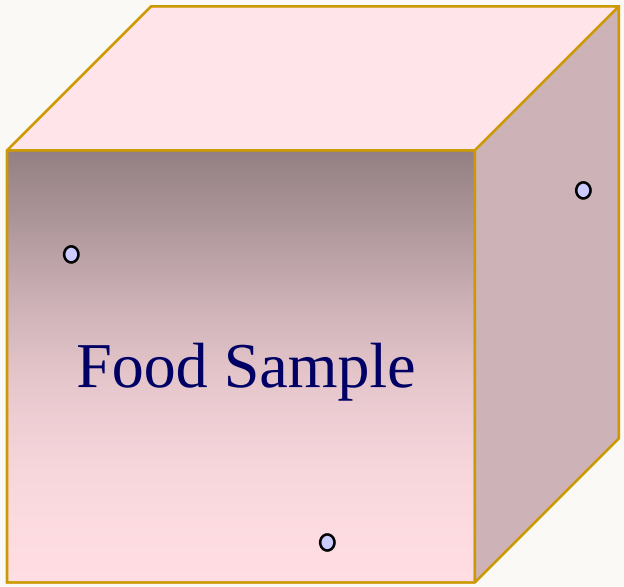
Wire-bond (with epoxy)

Micro-fluidic Tubes

PC board w. heater

Edge Connector

BioChip



10-100 mL

Dilute Cells

Challenge

>1000x size change

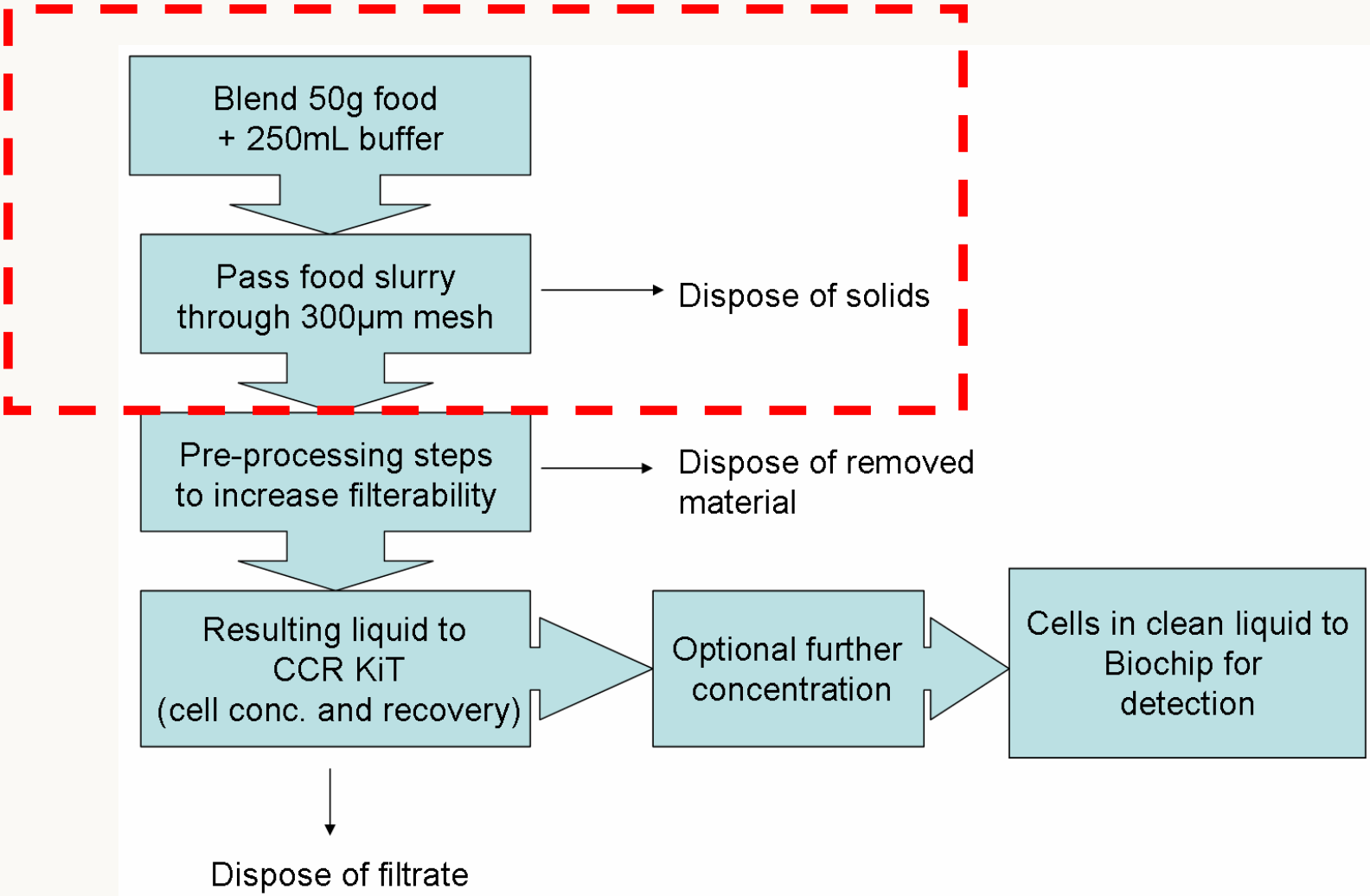
Detection volume

0.10-10 uL

Captured Cells

ERRC, 2000

General strategy for present work: Recovery of live bacteria



2. Extract Microorganisms

In order of decreasing severity

blender	**>**	**stomacher machine**	**>**	**massage**
2 min air (froth)		2 min in air		2 min in air
20°C		20°C		20°C
Decant into stomacher bag		In stomacher bag		In stomacher bag

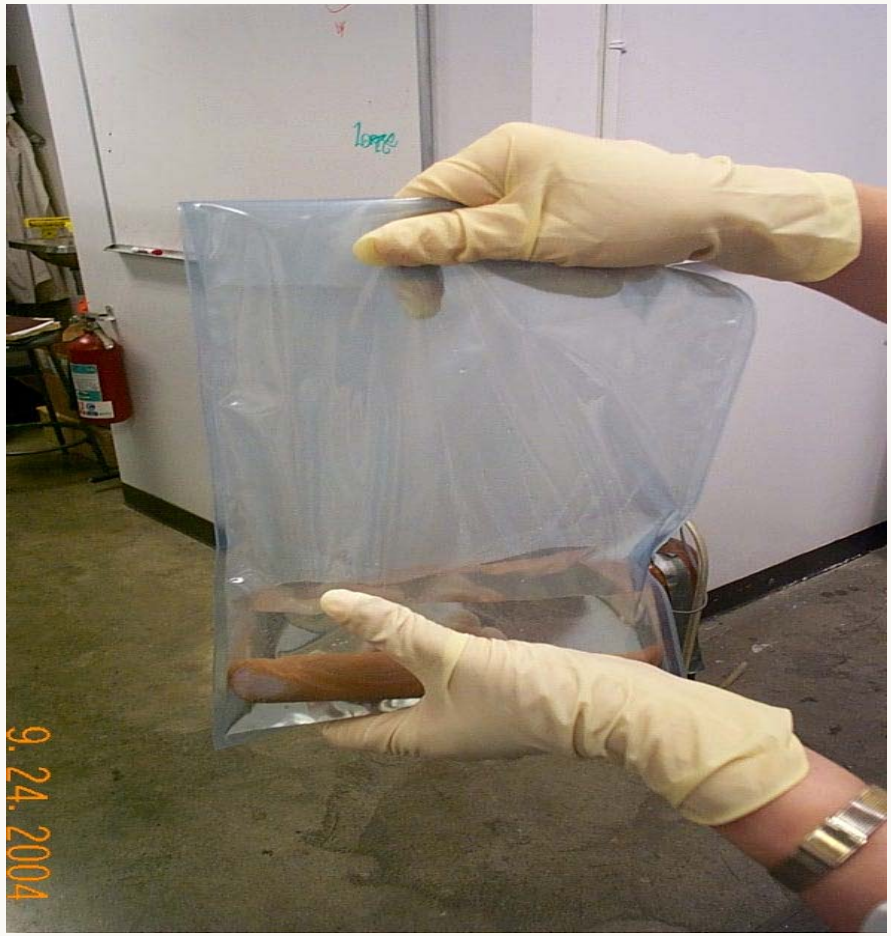
Homogenized Hotdog Experiment

Before blending the hotdog

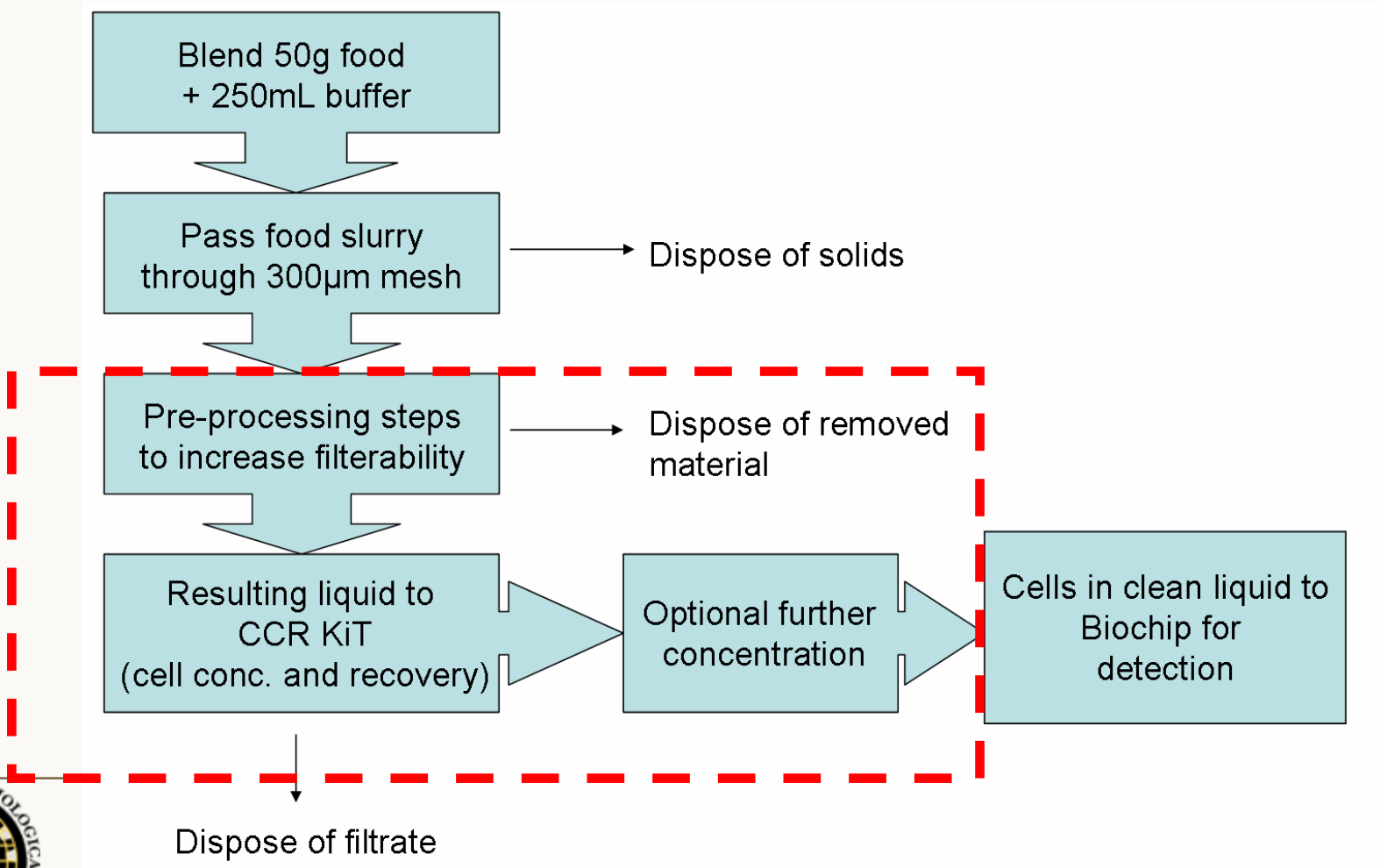
Homogenized Hotdog Experiment

After hotdog was blended

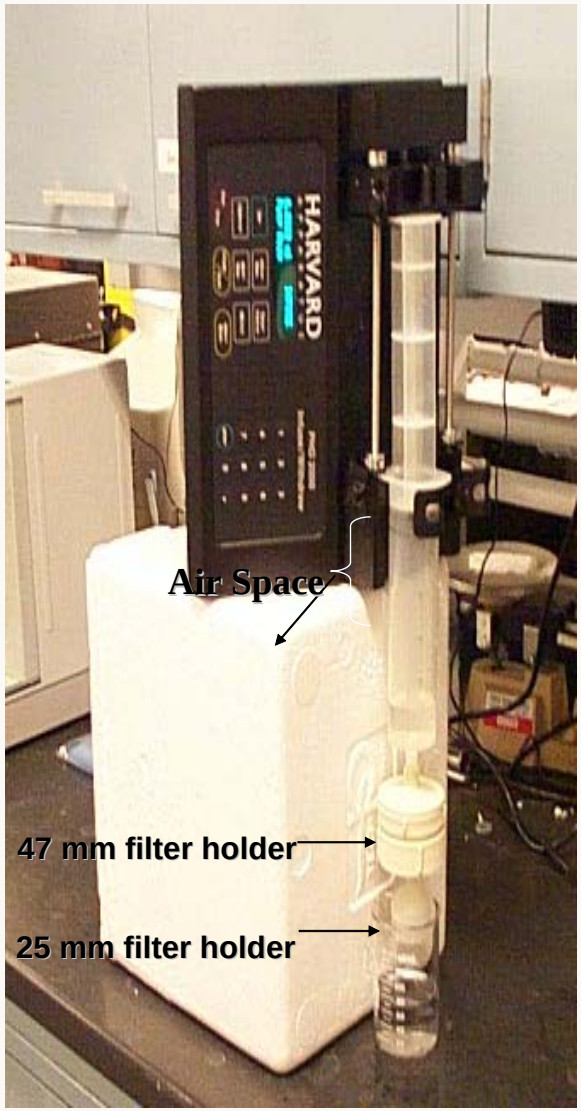
Massaged Hotdog Experiment



General strategy for present work: Recovery of live bacteria



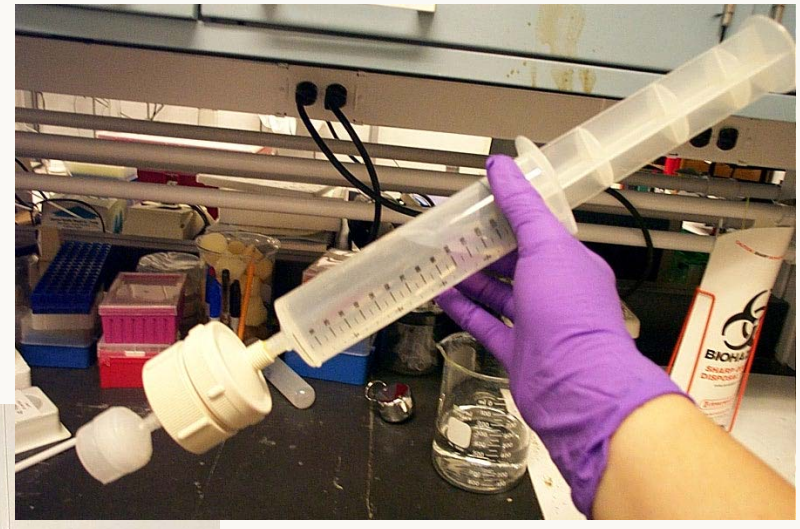
Cell Concentration and Recovery

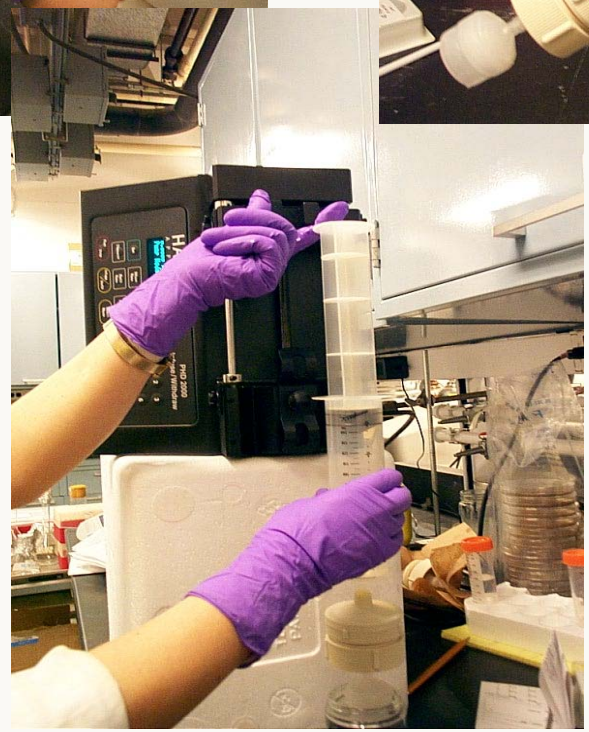


CCR Kit Assembly

- Microfiltration basis
- Designed to capture and recover bacterial cells from a food-derived sample
- Uses membranes in series to process 100 mL hot dog extract into 0.1 to 1 mL sample

CCR Method



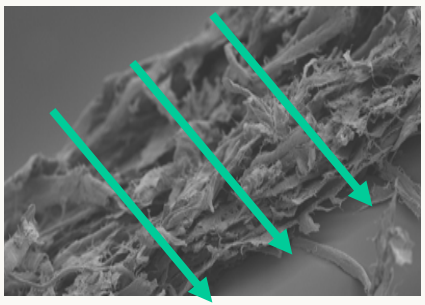


CCR Work Summary

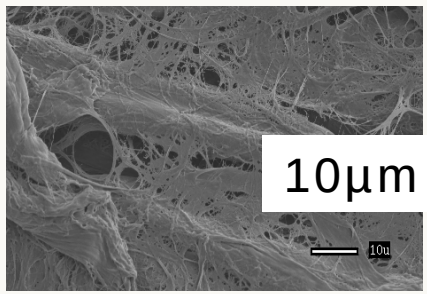
- Careful selection of membranes
  - Pore size and distribution
  - Surface chemistry
  - Interaction of target cell

SEM by Chia-Ping Huang

Side view

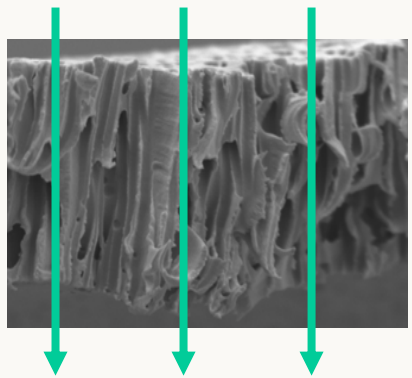


Top down

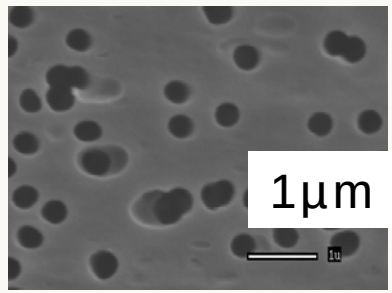


2.5µm depth filter – 47mm dia PRE-FILTER

Side view

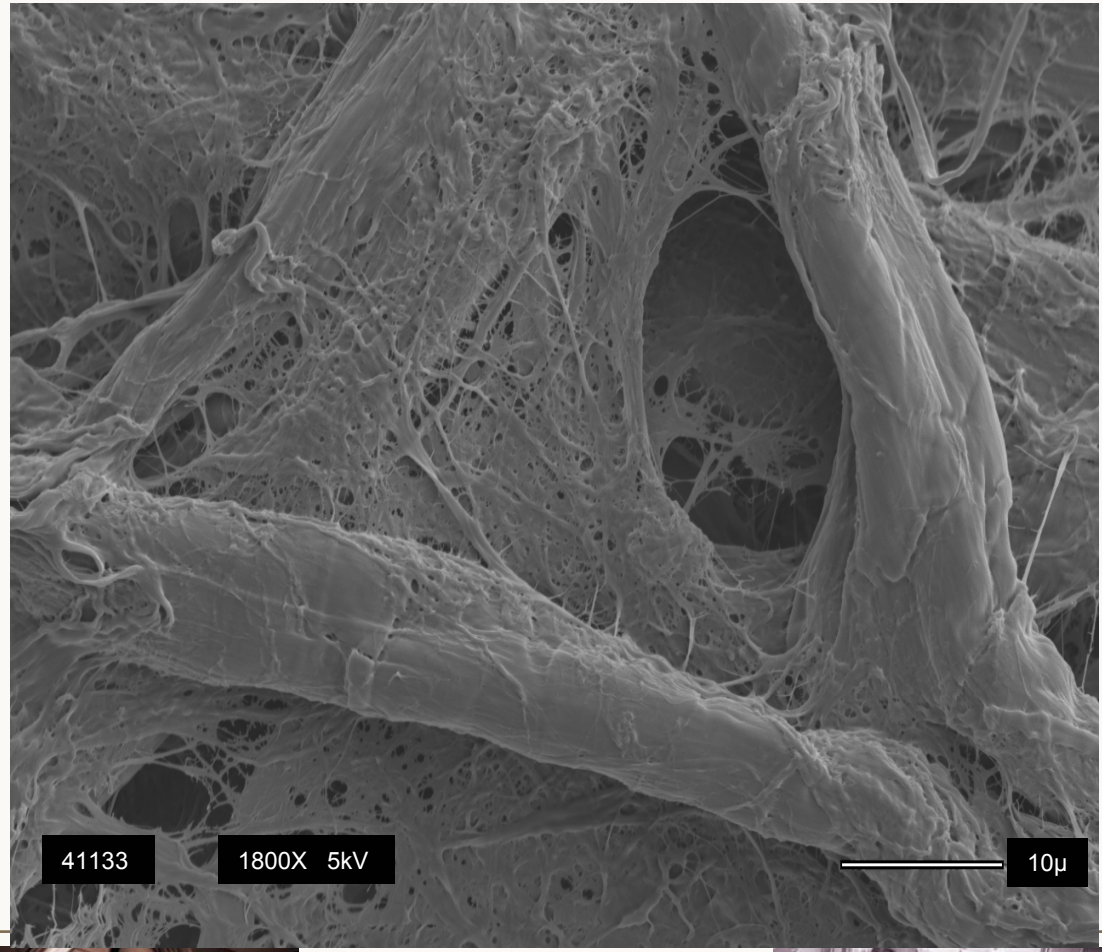


Top down



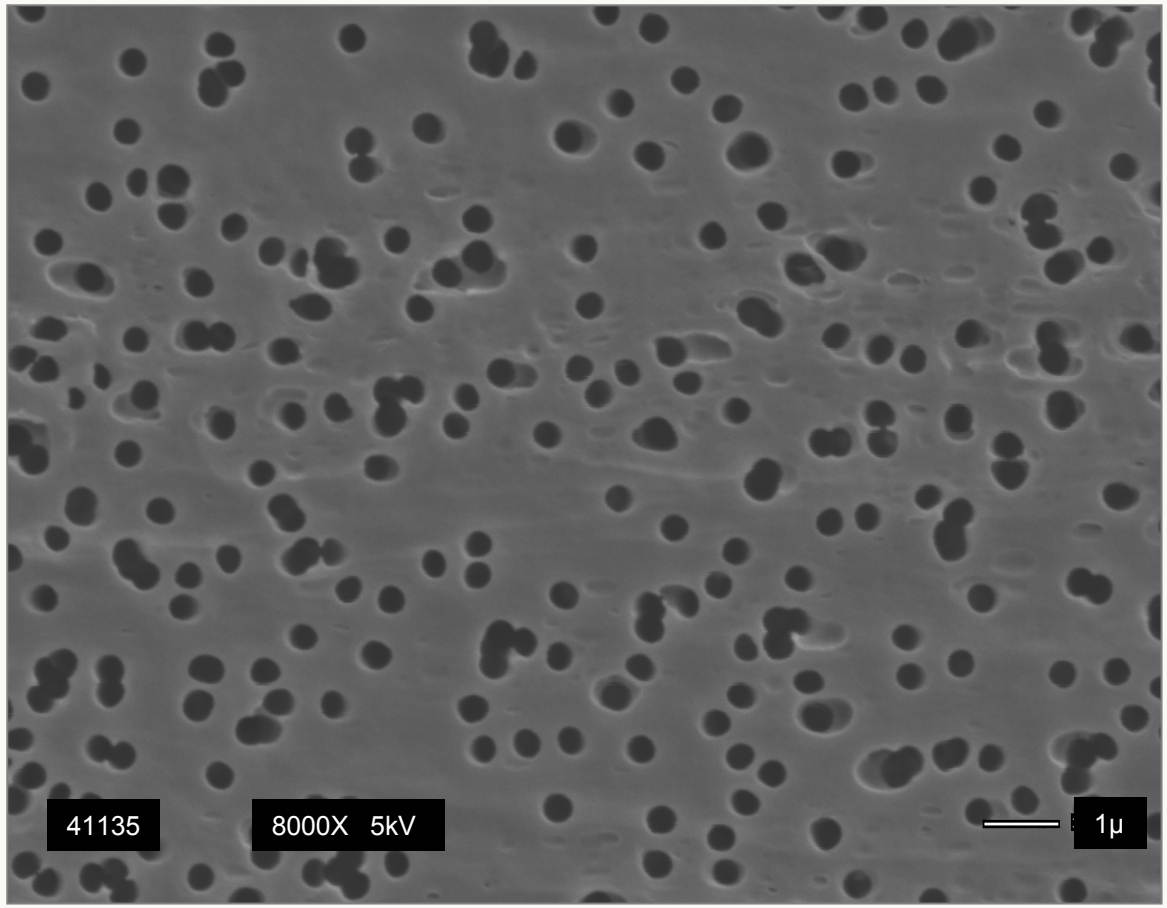
0.4µm screen filter – 25mm dia capture and conc. bacteria

Depth Filter Before Use



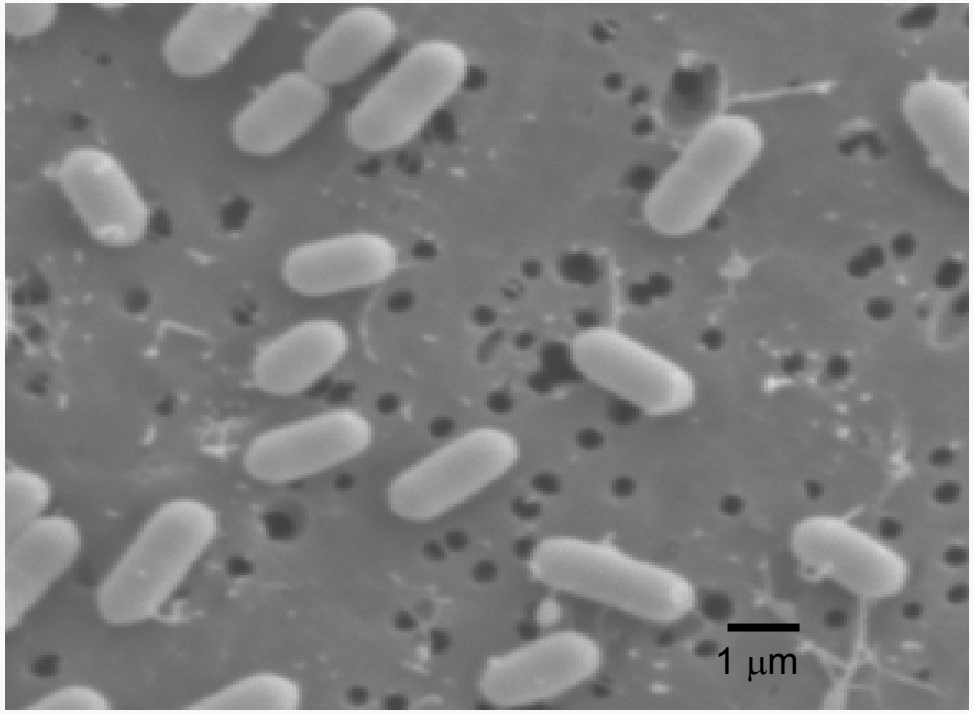
Whatman 42

Screen Filter Before Use



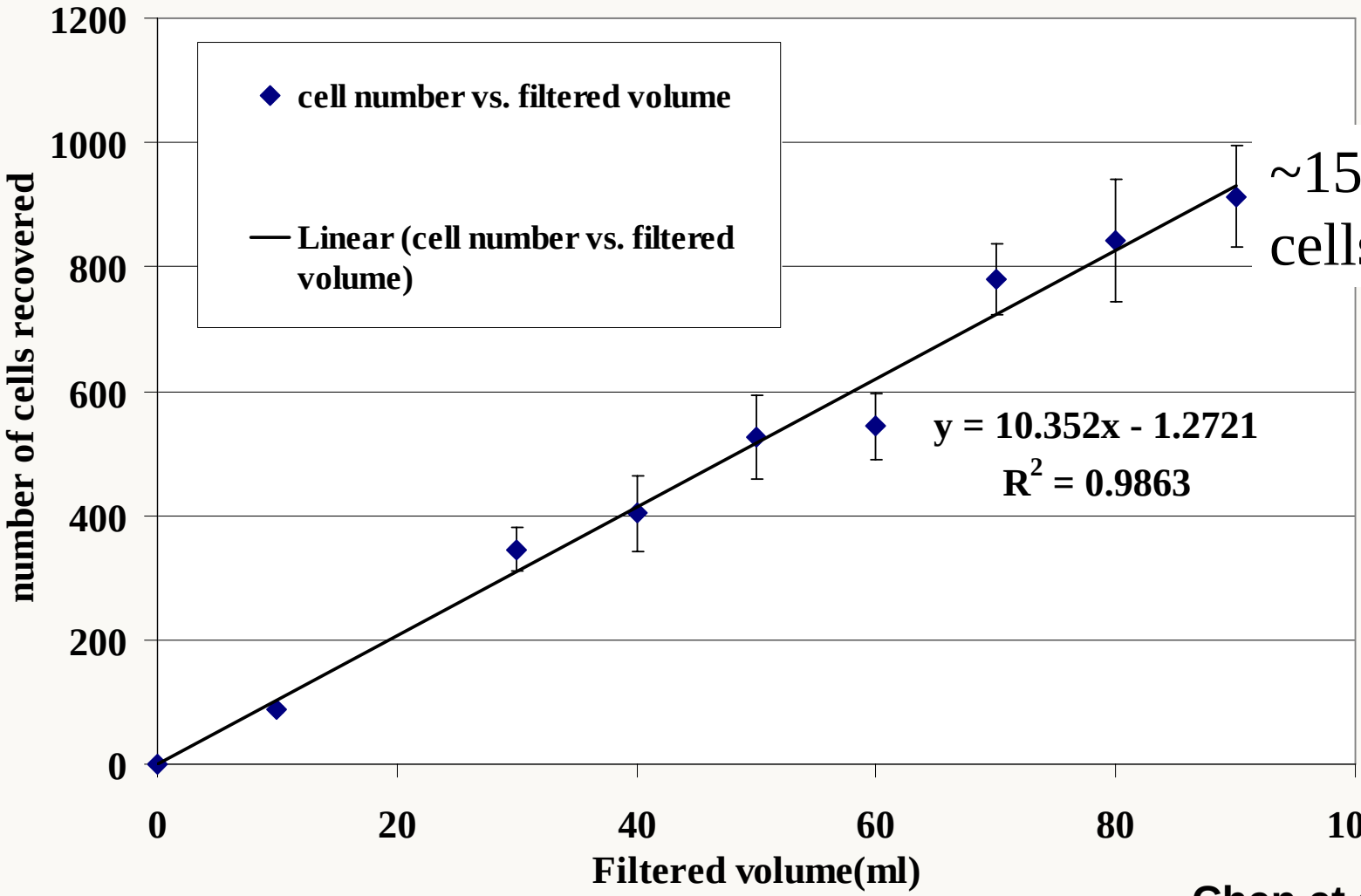
0.4 micron Polycarbonate

Membrane Properties



Polycarbonate membrane

Bacterial cells recovered in 500 µL from hot dog meat broth containing 15 cells/ mL



~1500 cells/mL

Chen et al, 2004

Validating Location of Cells

Need to “see” cells for rapidly screening different filtration and sensing systems:

led to use of GFP bacteria

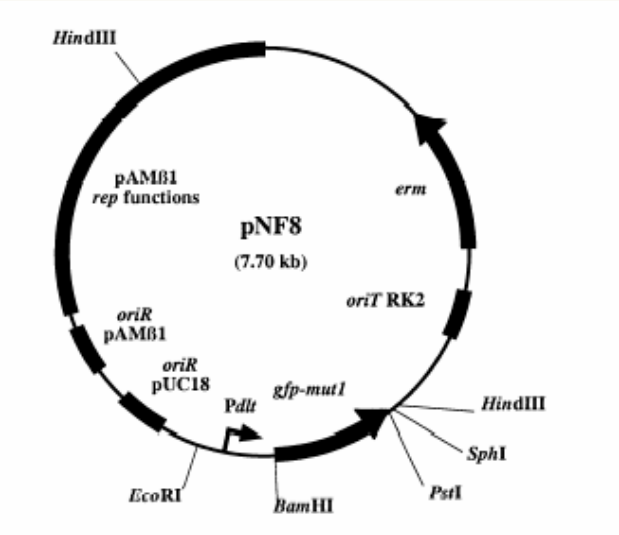
red or green *E. coli*

green *L. monocytogenes*

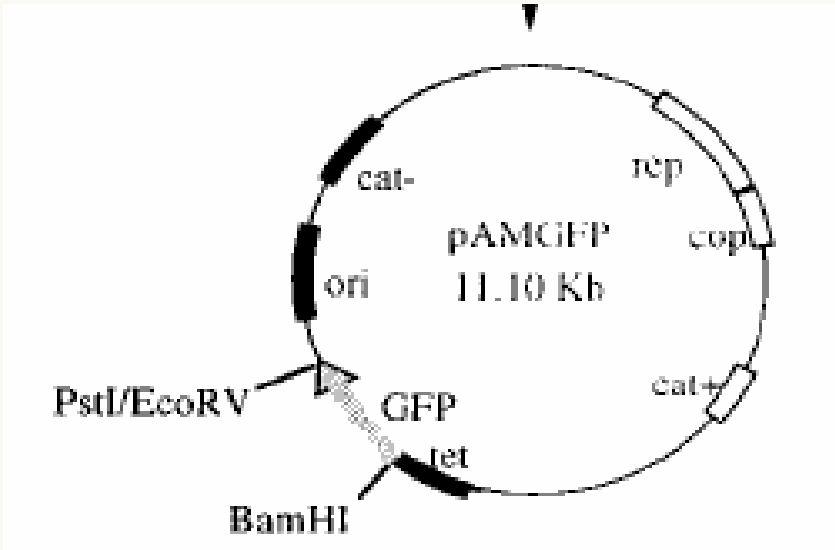
(Applegate and Sedlak, 2004)

Expressing Fluorescent Proteins

Fortineau *et al.* (2000) Res. Microbiol.



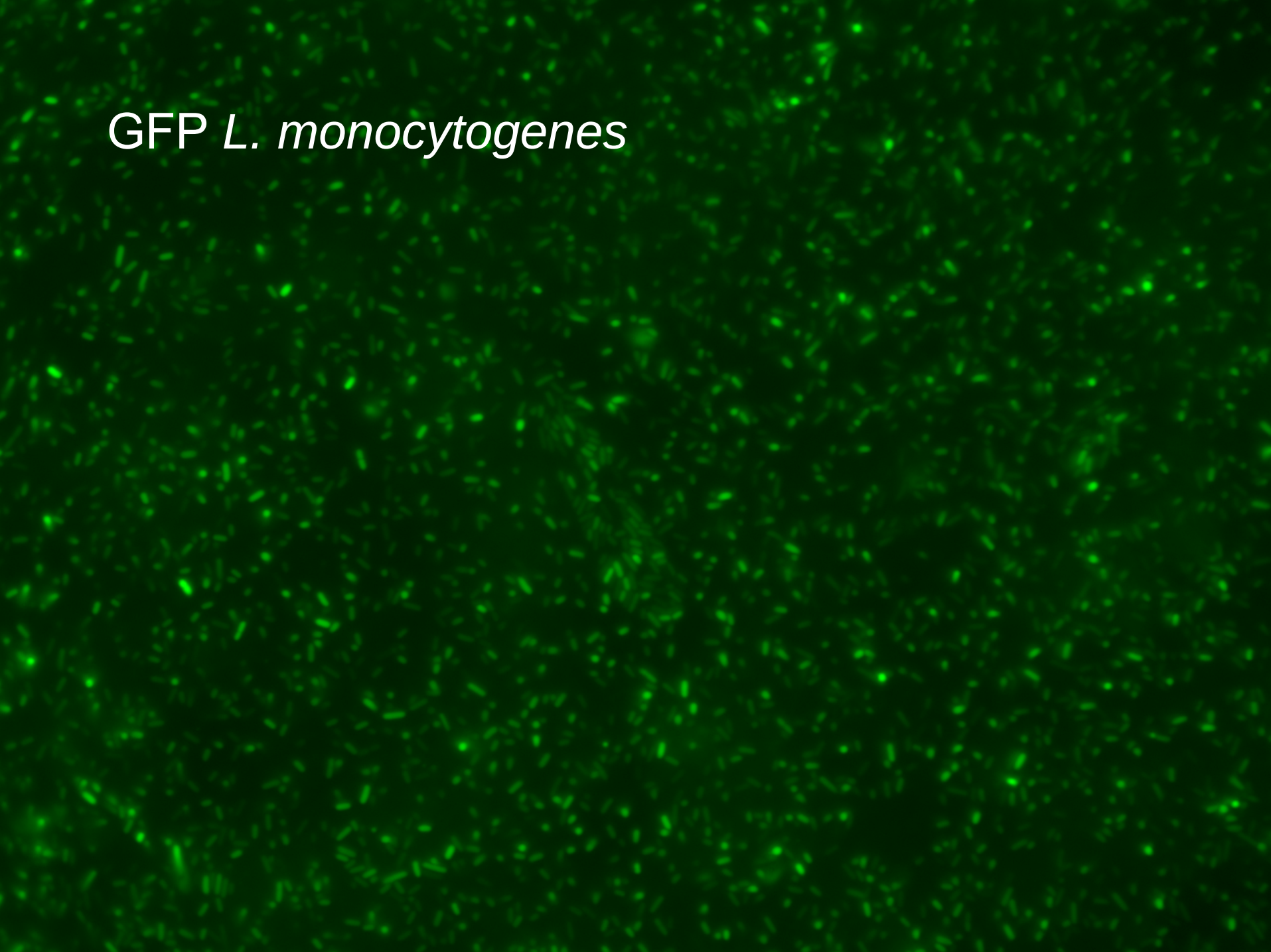
Wilson *et al.* (2001) Infect. Immun.

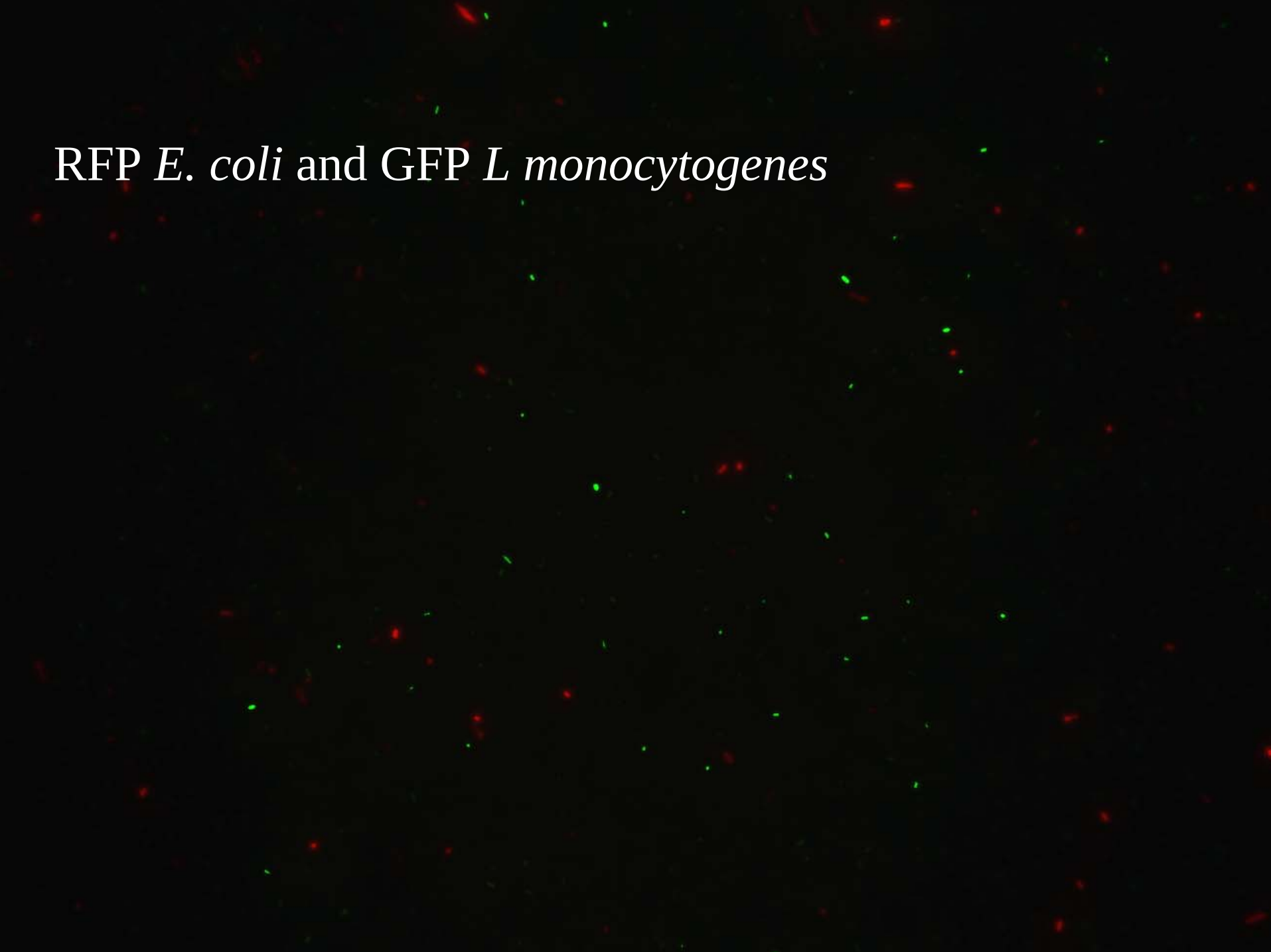


Listeria monocytogenes ATCC 23074

Mutant GFP protein – GFP-mut1

Mutant GFP protein – GFP-mut3


GFP *L. monocytogenes*


RFP *E. coli* and GFP *L monocytogenes*

Sampling Issue

› How high a concentration is needed in sample introduced to biochip to guarantee detection of cells?

› How much volume must be processed from hot dog sample to guarantee capture of at least one cell?

SYSTEM CHARACTERIZATION

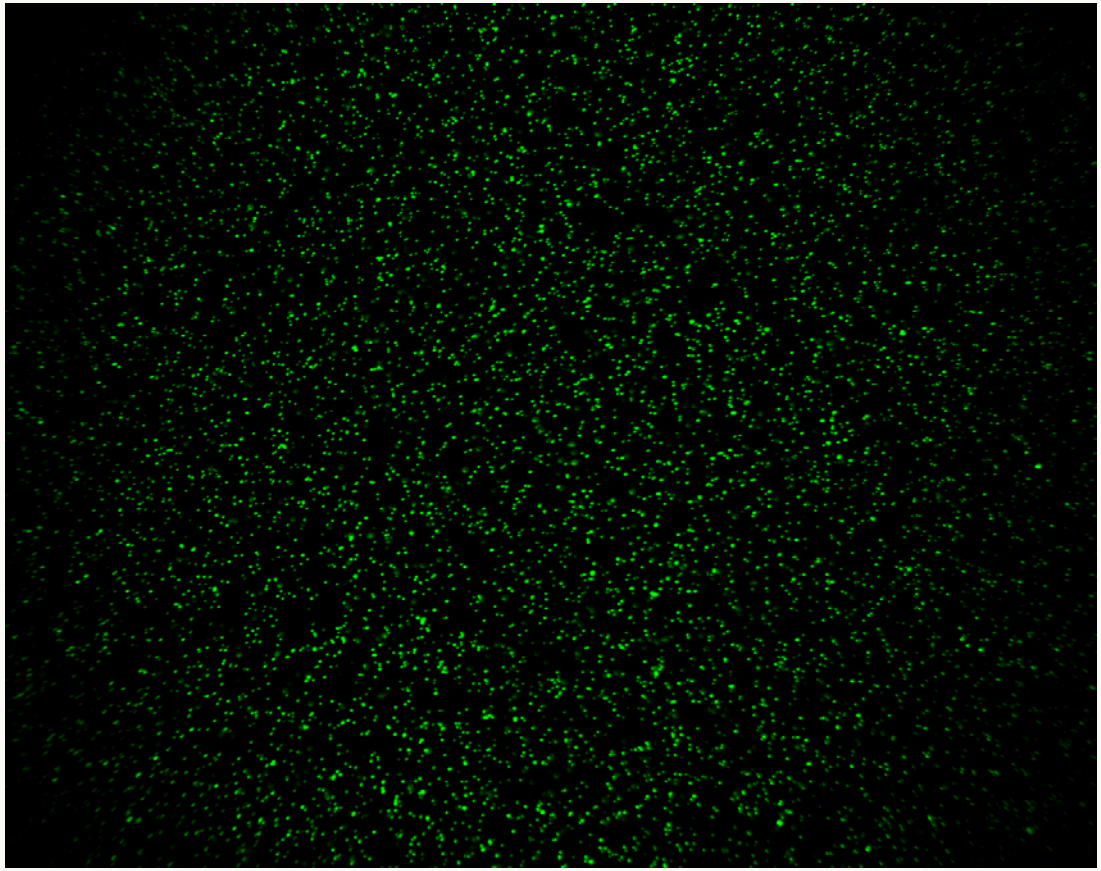
› Uneven distribution of microbes – Graininess;

› Uneven distribution of sample matrices.

› Variability

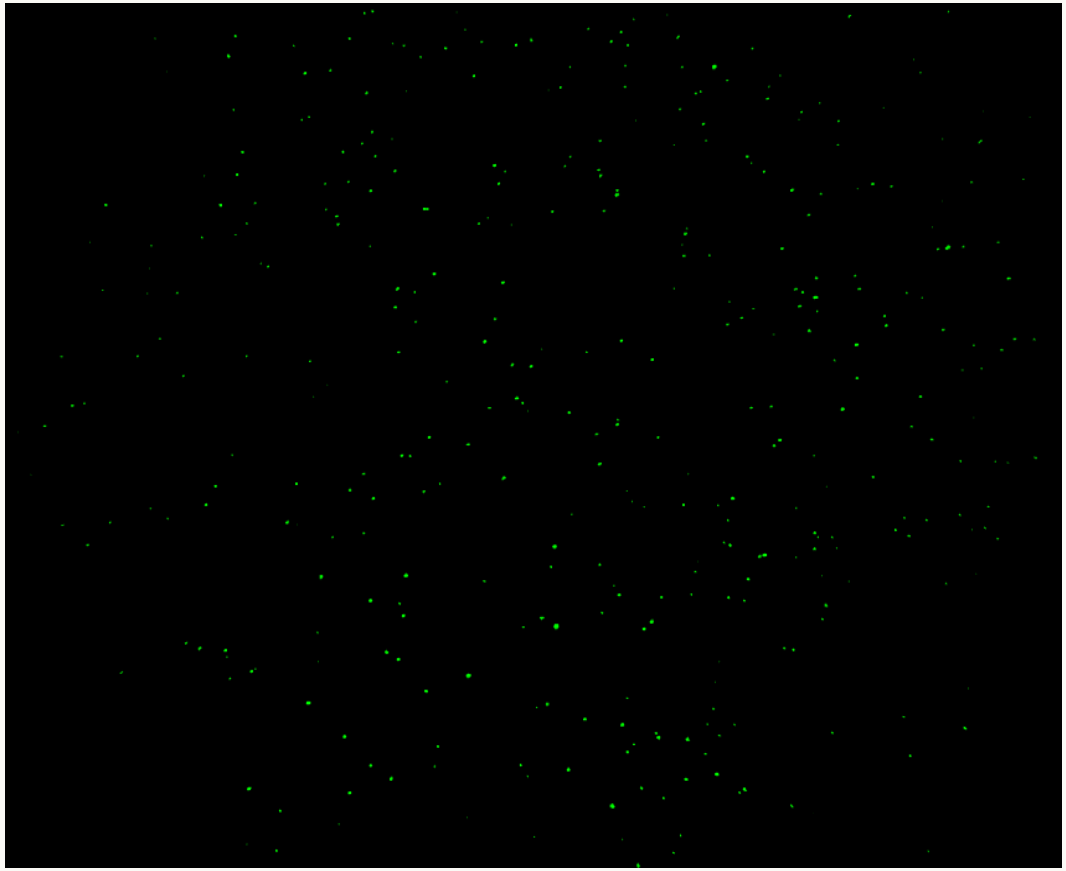
› Poor reproducibility

Distribution of GFP E. coli



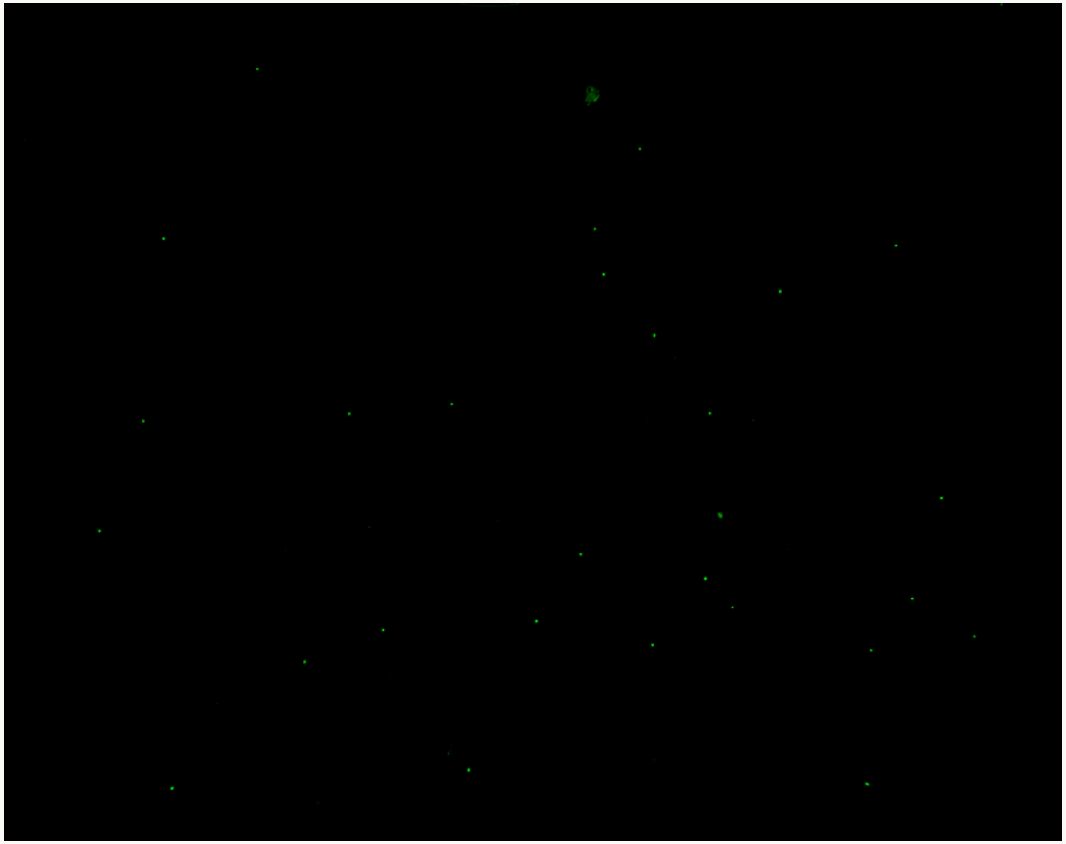
10^8 cells/mL
10X

Distribution of GFP E. coli



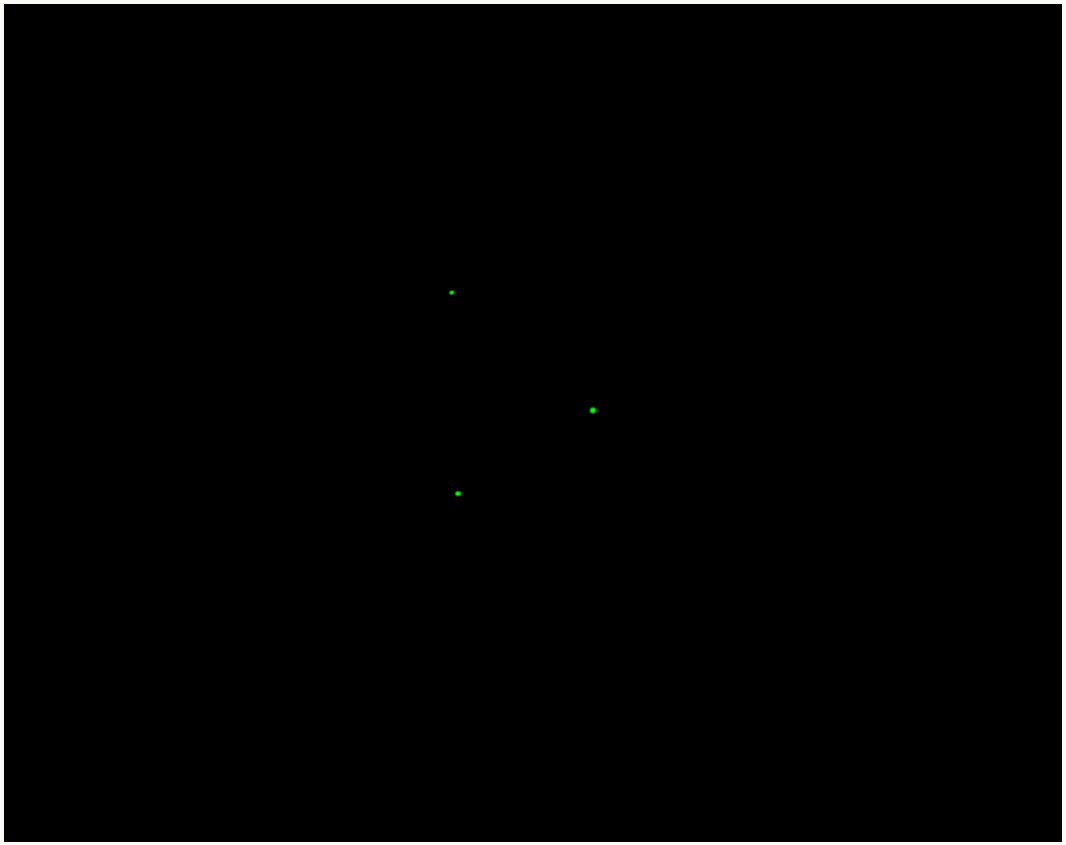
10^7 cells/mL
10X

Distribution of GFP E. coli



10^6 cells/mL
10X

Distribution of GFP E. coli



10^5 cells/mL
10X